World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.com/
Review Article

Patent review on cyclodextrin based nanosponges prepared by different methods: physicochemical characterization, factors influencing formation and applications

Pritesh Patel, Ashwini Deshpande*

SVKM's NMIMS, School of Pharmacy and Technology Management, Shirpur, Dist-Dhulia, Maharashtra-425405, India

Received: 06-03-2014 / Revised: 13-03-2014 / Accepted: 27-03-2014

ABSTRACT

Cyclodextrin nanosponges are solid, porous,biocompatible nanoparticulate three dimensional structures and have been used as delivery system of different drugs.Nanospongesare diminutive sponges with a size of about a virus. Specific sites are being targeted by tiny nanosponges in the body during circulation and release the drug in controlled expected manner.Porous insoluble nanosponges with a crystalline or amorphous structure andspherical shape can be formed by using different types of cyclodextrins.Different types of cyclodextrins, cross-linkers and degree of cross-linking between them can alter the dimension and polarity of the polymer mesh.Cyclodextrin-based nanosponges can enclose different types of lipophilic or hydrophilic drug molecules by forming inclusion complex. Inclusion Complexes formed between drug and nanosponges can be characterized by different methods like microscopy,solubility studies, zeta potential, differential scanning calorimetry, fouriertransform-infrared spectroscopy etc. Different factors like type of polymer, type of drug, temperature and method of preparation can affect formation as well as performance of nanosponges. Benefits ofcyclodextrin based nanosponges areaqueous solubilityimprovement, increment in stability period, maskingunpleasant flavours, preventing gastrointestinal irritationand obtainingsustained delivery systems or designinginnovative drug carriers for nanomedicine.

Key Words: cyclodextrin, nanosponges, cross-linker, inclusion complex, solubility, stability, drug carrier.

INTRODUCTION

Cyclodextrin-based nanosponges are biocompatible nanoparticles used for improvement in dissolution rate, solubility and stability of drugs, masking unpleasant flavors, converting liquid substances to solids and prolonging the release of drug. Mostly two methods used for the preparation of nanosponges are cross-linking reaction by condensation polymerization and cross linking reaction by interfacial phenomenon. Nanosponges prepared by polymerization reaction showed the promising results in anticancer drug delivery system, proteins delivery system, anti-inflammatory drugs and antifungal drug delivery system[1,2,3].

By using organic carbonates as cross-linkers, cyclodextrin based nanosponges are prepared for eliminating the chlorinated aromatic compounds present in water in traces[1,2,3]. For solubility enhancement, various formulation approaches like use of co-solvents, surfactants, inclusion complex formation, solid dispersion, hot melt extrusion, supercritical fluid technology etc. are adopted in which most interesting approach is inclusion complex formation of activeingredients and cyclodextrins by using cross-linker[3].Cyclodextrins (CD) areshaped like truncated cone structurein which arrangement of the functional groups of the glucose molecules is such that glucose molecule's upper surface is polar and inner cavity is lipophilic which facilitates CD's to form inclusion complexes that are stable in organic solutions of suitable size and polarity[3]. Inclusion complexes are generally prepared for solubilityimprovement of poorly water-soluble drug molecules, increment in stability period of drug molecules and masking unpleasant flavors[3]. These inclusion complexes carry lipophilic drug inside the hydrophobic cavity of CD's[1,2,3]. Only because of this reason cyclodextrin is having numerous applications in various fields like pharmaceutical, catalysis, analytical, cosmetics etc.

***Corresponding Author Address: Ashwini Deshpande,** Department of Pharmaceutics, School of Pharmacy &Technology Management, NMIMS, Shirpur, India; **Email ID:** ashwinideshpande4@gmail.com*

wherein the properties of the inclusion compounds are exploited[3]. Cross-linkers like Diisocyanates, epichlorohydrin or organic carbonates were used to get polymerization [3]. Nanosponges are covalently bound functionalised cyclodextrin systems which can be used as carriers of active ingredients. Because of these features, cyclodextrin based nanosponges can be used to solve the intrinsic problems of the active ingredient such as the protection,poor hydro-solubility, instability, gastric irritation and toxicity[1, 2, 3].

Nanosponges based on cyclodextrins can be obtained using organic carbonates as cross-linkers, operating in particular with the aid of ultrasounds without a solvent[3].Under sonication, cyclodextrin based nanosponges can beobtainedby a reaction between natural cyclodextrin (α,β and γ) and the diphenyl carbonate (DPC) or other organic carbonates without a solvent addition which can be operated between the ambient temperature and 90°C[3]. Cyclodextrin based nanosponges canalso synthesized by using organic carbonates which involves the use of solvents such as DMF or DMSO[3]. In which reaction is carried out at temperatures higher than 130-140°C and leads to the formation of a reticulate transparent block which has proved completion of polymerization reaction resulting inclusion complex formation[3].The product, which has suitable characteristics for its use as carrier of active ingredients, shows under the optical microscope a peculiar morphology: it is made up of spheroidal particles with regular dimensions smaller than 5 microns[1,2,3].**Table 1** shows list of different drug molecules complexed by nanosponges and **Table 2**shows list of chemicals(polymers and cross-linkers) used for synthesis of nanosponges.

Methods for Preparation of nanosponges:

1) ***Different methods for preparation of β-CD based nanosponges using Diphenyl Carbonate.***

Method 1[1,2,3]

In a flask mix0.001 mols of anhydrous β-CD and0.004 mols of diphenyl carbonate (DPC)in the molar ratio of CD:DPC=1:4.Subject this mixture for sonication in an ultrasound bathfilled with water at 90°Cfor 5 hrs.The reaction mixture is allowed to cool down. The resultant cross-linked transparent block is to be broken down roughly. Phenol crystals of fine needle shape get deposited on the flask's neck and part of the phenol developed helps the product to be agglomerated. Wash the product (roughly ground) with water for theremoval of non-reacted cyclodextrin, and additionallywash in Soxhlet's extraction apparatus with ethanol to ensure removal of unknown byproducts formed during reaction.A fine white powder, insolublein water and other organic solvents is obtained as final product at the end of reaction. This can be further confirmed by the supporting studies.

Method 2[1,2,3]

Same procedure, as mentioned in Method 1 up to sonication is to be employed.The reaction mixture is allowed to cool and then rotavapor is used to concentrate mixture in a small volume. Excess water is added in the end, followed by filtering and washing with water for a extensive period.A fine white powder, insolublein water and other organic solvents is obtained as final product at the end of reaction which is to be dried.

The same procedure can be repeated with molar ratio of CD: DPC 1:2

Method 3[1,2,3]

Mixture in the molar ratio of CD:DPC = 1:2 in a beaker is placed in an oil bath bain-marie under sonication and heated at 90°C for 4 h at 19kHz using an ultrasound probe capable of supplying a maximum power of 250W.The reaction mixture is allowed to cool down and break-down of the obtained product is roughly facilitated. For removal of the non-reacted cyclodextrin, the product is washed with water followed by washing in Soxhlet apparatus with acetone to eliminate the phenol developed and the residual DPC.A fine white powder which is insolublein water and other organic solvents is obtained as final product at the end of reaction.This is the same method can be repeated with molar ratio of CD: DPC=1:3 andCD:DPC=2:1.

2) ***Different methods for preparation of α,β and γ-CD based nanosponges using different cross-linkers****according to* **Table 3**

Factors influencing Nanosponge Formation:

Type of polymer: Type of polymer used has direct impact on the formation as well as the performance ofNanosponges.Hydroxy propyl β-cyclodextrin possess good affinity to form inclusion complex as compared to α, β and γ-cyclodextrin. Suitability of cavity size in inclusion complex confirm the accommodation of a drug molecule of particular size in nanopsonge[5].

Type of drugs: Complex of drug molecules with nanosponges should possess certain characteristics which are enlisted below[7] .

- Molecular weight should be in between 100 and 400 gm/mole.

- Drug molecule consists of less than five condensed rings.
- Solubility of drug molecule in water should be less than 10mg/mL.
- Melting point of the substance should be below 250°C.

Temperature: Temperature change can play vital role in affecting Drug/Nanosponge complexation. In general, the magnitude of stability constant of the Drug/Nanosponge complex decreases with increase in the temperature which may be due to reduction in drug/nanosponge interaction forces [(8)].

Method of preparation: Nanosponges can be prepared by different methods like polymerization condensation, interfacial polymerization, emulsion solvent diffusion, ultrasound etc.Drug/Nanosponge complexation can be affected by method of loading the drug into the nanosponge. Nature of the drug, polymer and temperature can affect the effectiveness of a method of preparation and in most cases of nanosponge formation, drug complexation was effectively obtained by freeze drying.[(9)].

Degree of substitution: The nanosponge complex forming ability may be greatly affected by type, number and position of the substituent on the parent polymeric molecule. Number of substitutions present is directly correlated to the degree of crosslinking. As the number of substituents increases, the probability of crosslinking increases correspondingly. Highly porous nanosponges can be obtained due to higher degree of cross-linking resulting in formation of a mesh type network[(9)].

Characterization of Nanosponges:

Solubility Studies: Phase solubility study is most widely used approach for inclusion complexation which scrutinizes the effect of a nanosponge on the solubility of the drug. Nanosponges are confirmed by insolubility in water and organic solvents like DMF(Dimethyl Formamide) and DMSO (Dimethyl Sulfoxide) [(5)].

Loading efficiency: Disperse the drug loaded nanosponges in the solvent in which drug is soluble,sonicate for 5 min to break the complex, diluted with suitable solvent andanalyze applying suitable method [(5)].

FT-IR(Infrared) spectroscopy: FTIR spectra of pure drug, β-cyclodextrin, nanosponge formulation can be compared to understand interaction between pure drug and nanosponge complex by KBr pellet method using a FT-IR spectrometer, at specific wavelength[(5,10,11)].

Zeta potential: Zeta potential is widely used for quantification of the magnitude of the electrical surface charge at the double layer. The significance of zeta potential is that its value can be related to the stability of formulation. More than 30 mV zeta potential value in water indicates good stability of Nanosponge.It can be measured by using zetasizeror particle size equipment[(10)].

Microscopy studies: For morphological study (particle size, shape) of drug, nanosponges and product(drug/nanopsponge complex), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) can be used. Formation of the inclusion complexes can be confirmed by observing difference in crystalline state of pure drug, cyclodextrin and product under electron microscope[(10, 11)].

Thin Layer Chromatography (TLC): TLC can also denote the complex formation. On running the sample of pure drug and nanosponges complex on TLC plate, the retention factor(R_f) was changed as compared to pure drug which confirms the complex formation [(11)].

Differential scanning calorimetry (DSC): The thermal behaviour of drug-CD complex can be studied using DSC in order to confirm the formation of complex. DSC thermogram of pure drug shows an endothermic peak at its melting point. This peak for drug will be absent in DSC thermogram of nanosponge[(12)].

In vitro release studies: In vitro release of drug from nanosponge formulations confers the release behaviour of the drug from nanosponges. Suitable dissolution and analysis method can be employed based on drug and type of formulation as per the aim of the study[(12)].

Accelerated Stability Studies: As stability is the main issue related to drug product, these studies are mandatory to be performed. Charge freshly prepared formulation in stability chamber as per the ICH guidelines and study is to be performed[(12)].

APPLICATIONS OF NANOSPONGES [(4,5)]:

Solubility Enhancement: One of the key hindrance to the formulation and development in pharmaceuticals is poor solubility of drugs in water. To solve the problem of solubility, numerous approaches have been investigated. Inclusion complex of cyclodextrin approach is widely used in pharmaceutical field for

improvement of solubility and bioavailability of lipophilic drugs. Nanosponges can enhance the wetting and solubility of poorly soluble molecules[4]. Swaminathan*et al*. studied a nanosponge formulation of **itraconazole**having solubility of 1 ng/mL at physiological pH. Nanosponges improved the solubility of the itraconazole by over 27-fold; and on adding PVP solubility was increased by 55-fold. Thus Nanosponge formulation could increase solubility and the bioavailability of itraconazole[13].

Nanosponges for Drug Delivery: The nanosponges can be formulated as Oral, Parenteral and Topical dosage forms. For the oral administration, capsules or tablets can be prepared by dispersion of nanosponge complex in a matrix of excipients, diluents and lubricants. For the parenteral administration, sterile water, saline or other aqueous solutions are used as carrier for nanosponge complex. For topical administration, nanosponge complex is incorporated in topical hydrogel[4].

Vavia prepared nanosponges of **nelfinavirmesylate** to improve dissolution rate, solubility and ultimately bioavailability of the drug[17]. Nelfinavir is a HIV-1 and HIV-2 protease inhibitor which used forthe treatment of HIV infections.From nanosponges, found drug release rate was less than from a β-CD complex which shows that nanosponges were proposed as a sustained drug delivery system for oral administration[17].

Acyclovir is a drug withpoor bioavailability, medium polarity andsolubility of 1.5 mg/mLin water. Special carboxylated nanosponges, enclosing dissociable carboxylic groups in their structure were developed for its encapsulation[9]. In addition to the cyclodextrin cavities, they also represent additional electrostatic contribution for encapsulation of drug. Carboxylic groups present in the nanosponge structure and the amino group of acyclovir may show some electrostatic interactions.

Rao*et al*. studied that **telmisartan**affected by carbonate nanosponges.Telmisartan is an angiotensine II receptor antagonist, antihypertensive BCS class II drug andhaving solubility of 9.9 µg/ml in water which ultimately lowersthe bioavailability. The formation of complex of telmisartan with nanosponges and $NaHCO_3$ was observed synergistically to enhance the dissolution rate as well as bioavailability of telmisartan[23].

Nanosponges as carriers to protect molecules form light or degradation: Alternative use for nanosponges is as carriers to safeguard encapsulatedmolecules from light or from degradation (chemical or enzymatic). Light-sensitive model drug **5-fluorouracile**was used for evaluation of the potential protection application**.** Up to 30% of 5-fluorouracile can be incorporated by β-CD nanosponges formation [18]. Furthermore, encapsulation of 5-fluorouracile in nanosponges protected the drug and maintained its cytotoxicity against MCF-7 cells.

Another example was the cyclodextrin based nanosponges of **camptothecin** [10]. The anti-tumour activity of camptothecin has been widely studied in both hematological and solid malignancies. Due to its poor solubility and high chemical instability, the lactone ring of the molecule is very susceptible to hydrolysis at physiological pH. The encapsulation of camptothecin in nanosponges was used to extend the shelf life as well as release of the drug. The nanosponges improved solubility of large amounts of the drug and protected the lactone ring from opening due to its high inclusion abilities and thereby increasing stability[4].

***Nanosponges as carrier for biocatalysts and in release of enzymes, proteins, vaccines, antibodies*:** For carrying enzymes and proteins a number of systems have been developed, for example, nanoparticles,microparticles, liposomes and hydrogels. It has been found that Cyclodextrin based nanosponges are predominantly appropriate carrier to adsorb enzymes, antibodies, proteins, and macromolecules. Specifically when enzymes are used, nanosponge formation can maintain their activity, efficiency, extend their operation, pH and temperature range of activity and allows the conduct of continuous flow processes. Furthermore, proteins and other macromoleculs can be carried by adsorbing or encapsulating them in cyclodextrin nanosponge. Swellable cyclodextrin-based nanosponges were purposely prepared for protein delivery by using a different synthetic routes[5].

New swellable cyclodextrin-based **poly (amidoamine)** nanosponges (PAA-NS) were synthesised by cross linking β-cyclodextrins with either short polyamido-amine chain or 2,2-bis(acrylamidoacetic acid)[9]. Swellable nanosponges were discovered to be sensitive to the pH of the emcompassing media. By using the high pressure-homogenisation technique, PAA-NS were abridged in nanosuspensions. For study of encapsulation capacity of these new nanosponges, bovine serum albumin (BSA) was used as a model protein. For achievement of high protein complexation capacity, nanosponges with BSA-

encapsulation efficiency greater than 90% were used. By forming carbonate nanosponges, encapsulation of albumin and its prolonged release was obtained[9].

Other applications of nanosponges: Even at very low concentrations nanosponges based on cyclodextrins can strongly bind organic molecules and remove them from water [19]. The identical notion can be suitable for eradication of bitter components from grape fruit juice by careful combination of polymer and cross-linker. The microporous hyper cross linked nanosponges have been used in selective separation of inorganic electrolytes by size exclusion chromatography. Vital role would be played by three dimensional nanosponges in the fractionalization of peptides for proteomic applications[20]. Nanosponges can be used as **carrierfor gases** like oxygen and carbon dioxide. Many biomedical applications can also take advantage of these nanosponges. Specifically, oxygen to the hypoxic tissues which are present in various diseases could be supplied by oxygen-filled nanosponges[21]. For the diagnosis purpose, nanosponges can soak up biomarkers selectively.[1]

CONCLUSION

Cyclodextrin based nanosponges possess different properties like encapsulation ability, unpleasant flavors masking, biocompatibility, solubilisation capacity and stabilization capacity which give benefits to poorly soluble BCS class II drugs. By controlling the ratio of polymer to cross-linker and drug to nanosponge complex, particle size and drug release can be modified. Different dosage forms like oral, parenteral and topical can be developed for both types of drug molecules like lipophilic as well as hydrophilic because of spherical shape and tiny sizeof nanosponges. Nanosponges can broaden the range of applications like solubility enhancement, for drug delivery, as carrier for biocatalyst and in release of enzymes, proteins, vaccines, antibodies etc.For the delivery of poorly soluble active drug molecules, cyclodextrin based nanosponges can be considered as multifunctional nanoscale drug delivery system in nanoscience.

Table 1: DRUG MOLECULES COMPLEXED BY NANOSPONGES[4]

Drug	Therapeutic activity	Administration Route
Dexamethasone	anti-inflammatory	Oral , Parenteral
Flurbiprofen	anti-inflammatory	Oral
Doxorubicin	Antineoplastic	Parenteral
Progesterone	Hormonal	Oral
Itraconazole	Antifungal	Oral , Topical
5-fluorouracile	Antineoplastic	Parenteral ,Topical
Tamoxifen	Antiestrogen	Oral
Resveratrol	Antioxidant	Topical
Paclitaxel	Antineoplastic	Parenteral
Camptothecin	Antineoplastic	Parenteral
Omeprazole	Antiulcerative	Oral
Nelfinavirmesylate	Antiviral	Oral
Acetylsalicylic acid	Analgesic	Oral
Acyclovir	Antiviral	Topical, Parenteral
Gamma-oryizanol	Antioxidant	Topical
Telmisartan	Antihypertensive	Oral

Table 2: CHEMICALS USED FOR SYNTHESIS OF NANOSPONGES[5]

Polymers	Crosslinkers
Hyper cross linked Polystyrenes	Carbonyldiimidazoles
Cyclodextrines and its derivatives like Methyl β-Cyclodextrin	Diphenyl Carbonate
Alkyloxycarbonyl Cyclodextrins	Diarylcarbonates
2-Hydroxy Propyl β-Cyclodextrins	Diisocyanates
Copolymers like Poly(valerolactone-allylvalerolactone)	Pyromellitic anhydride, Epichloridrine, , Glutarldehyde
Poly(valerolactone-allylvalerolactoneoxepanedione)	Carboxylic acid dianhydrides
Ethyl Cellulose	2,2- bis(acrylamido) Acetic acid
PVA	Dichloromethane

Table 3 Different formula for preparation of nanosponges[6]

Aqueous Phase	Organic Phase	Dextrin Type	Cross-linking agent	Drug: Cross-linker Molar Ratio
0.1 M KOH	CH_2Cl_2	β-CD	CDI	1:8
0.1 M KOH	Methyl Isobutyl Ketone	β-CD	CDI	1:8
0.1 M NaOH	CH_2Cl_2	β-CD	CDI	1:8
0.1 M KOH	CH_2Cl_2	β-CD	CDI	1:8
0.1 M KOH	Hexane	β-CD	PMDA	1:8
0.1 M KOH	CH_2Cl_2	β-CD	TPh	1:2
0.1 M KOH	CH_2Cl_2	β-CD	TPh	1:4
0.1 M KOH	DMC	β-CD	DMC	1:8
0.1 M KOH	CH_2Cl_2	α-CD	CDI	1:8
0.1 M KOH	CH_2Cl_2	γ-CD	CDI	1:8
0.1 M LiOH	CH_2Cl_2	β-CD	CDI	1:8
0.1 M KOH	CH_2Cl_2	β-CD	DPC	1:8
0.1 M KOH	CH_2Cl_2	α-CD	CDI	1:8
0.1 M KOH	CH_2Cl_2	γ-CD	CDI	1:8
0.1 M KOH	Hexane	β-CD	PMDA	1:8
0.1 M KOH	CH_2Cl_2	β-CD	TPh	1:2
0.1 M KOH	CH_2Cl_2	β-CD	TDI	1:8
0.1 M KOH	CH_2Cl_2	β-CD	HDI	1:8
0.1 M KOH	CH_2Cl_2	β-CD	TDI	1:8
0.1 M KOH	CH_2Cl_2	β-CD	CDI	1:8
0.1 M KOH	CH_2Cl_2	β-CD	Epichlorohydrin	1:8
0.1 M KOH	CH_2Cl_2	β-CD	Epichlorohydrin	1:8

***PMDA**-Pyromellitic anhydride, **TPh**- Triphosgene, **CDI**-Carbonyl Di-Imidazole, **SSC**-SebacoylChloride ,**DCM**-Dicholomethane, **DPC**-Diphenylcarbonate, **DMC**-Dimethyl carbonate, **HDI**-Hexamethyldiisocyanate*

REFERENCES

1. TrottaF,Cavalli R, Tumiatti W, Zerbinati O, Roggero C, Vallero R. Ultrasound assisted synthesis of cyclodextrin based nanosponges. EP 1,632,503 A1, March 08, 2006.
2. TrottaF,Cavalli R, Tumiatti W, Zerbinati O, Roggero C, Vallero R. Ultrasound assisted synthesis of cyclodextrin based nanosponges. US 2008/0213384 A1,September 04,2008.
3. TrottaF,Cavalli R, Tumiatti W, Zerbinati O, Roggero C, Vallero R. Ultrasound assisted synthesis of cyclodextrin based nanosponges. WO 2006/002814 A1, January 12, 2006.
4. Trotta F,Zanetti M, Cavalli R. Cyclodextrin based nanosponges as drug carriers. Beilstein Journal of organic chemistry 2012; 8: 2091-2099
5. Selvamuthukumar S et al. Nanosponges:A novel class of drug delivery system-Review. J. Pharm Pharmaceutic Sci. 2012; 15(1): 103-111.
6. Trotta F, Shende P, Blasizzo M. Method for preparing dextrin nanosponges. WO 2012/147069 A1, November 1, 2012.
7. Rajeswari C et al. Cyclodextrins in drug delivery: an update review. AAPS pharmSciTech. 2005; 6(2): E329-E357.
8. Rao M et al.J. Inclusion Phenom. MacrocyclicChem., doi:10.1007/s10847-012-0224-7(in press).
9. Lembo D et al. Encapsulation of Acyclovir in new carboxylatedcyclodextin based nanosponge improves agent's antiviral efficacy. International Journal of Pharmaceutics 2013; 443: 262-272.
10. Swaminathan S et al. Cyclodextrin-based nanosponges encapsulating camptothecin: Physicochemical characterization, stability and cytotoxicity. Eur J Pharm Biopharm. 2010; 74: 193-201.
11. Ramnik S et al. Characterization of Cyclodextrin Inclusion complexes –A Review. J. Pharm Sci Tech. 2010; 2(3):171-183.
12. Shende P et al. Novel cyclodextrin nanosponges for delivery of calcium in hyperphosphatemia, InternationalJournal of Pharmaceutics, 2013, 7-8.
13. Swaminathan S et al. Inclusion Phenom. Macrocyclic Chem. 2007;57*:* 89–94.
14. Gelderblom H, et al. Eur. J. Cancer. 2011,37:1590–1598.
15. Mognetti B et al.J. Inclusion Phenom. Macrocyclic Chem. 2012;74*:* 201–210.
16. Cavalli R, Trotta F, Tumiatti WJ. Inclusion Phenom. Macrocyclic Chem. 2006; 56:209–213.
17. Vavia PR et al. Applications of nanosponges in drug delivery.InProceedings XIII InternationalCyclodextrin Symposium, Turin, Italy May 14–17, 2006; 207.
18. Cavalli R et al. 5-Fluorouracile loaded β-cyclodextrin nanosponges: In-vitro characterization and cytotoxicity. In Proceedings XIII InternationalCyclodextrin Symposium, Turin, Italy, May 14–17, 2006; 207.
19. Trotta F, Tumiatti W. Cross-linked polymers based on cyclodextrins for removing polluting agents. WO 03/085002 A1, 2003.
20. Wong VN et al. Separation of peptides with polyionic nanosponges for MALDIMS analysis. Langmuir, 2009; 25(3): 1459-1465.
21. Cavalli R et al. Nanosponge formulations as oxygen delivery systems. Int J Pharm, 2010; 402(1-2): 254-257.
22. Torne S, DarandaleS, Vavia P.Pharm. Dev. Technol.2012;1–7.
23. Amber Vet al. Cyclodextrin based novel drug delivery systems. J. Inclusion Phenom. Macrocyclic. Chem, 2008; 62:23-42.
24. Jenny A et al. Role of β- cyclodextrin nanosponges in polypropylene photooxidation Carbohydrate Polymers, 2011; 86: 127– 135.
25. Ranucci E et al. Cross-linked Resins by Stepwise Polyaddition of β-Cyclodextrin with Bis-acrylamides and Assessment of Their Potential as pH-Sensitive NPs for Site-Specific Protein Delivery, In Proceedings CRS 2012, Quebec City, Canada, July 15–17, 2012; 99.
26. Gilargi G, Trotta F, Cavalli R, Ferruti P, Nardo G, Roggero CM, Tumiatti V. Cyclodextrin nanosponges as a carrier for biocatalysts and in the delivery and release of enzymes,proteins,vaccines and antibiotics. WO 2009/149883 A1, December 17, 2009.
27. Trotta F, Cavalli R, Roggero C, Mognetti B, Berta GN. Cyclodextrin based nanosponges as a vehicle for antitumoral drugs. WO 2009/003656 A1, January 08, 2009.